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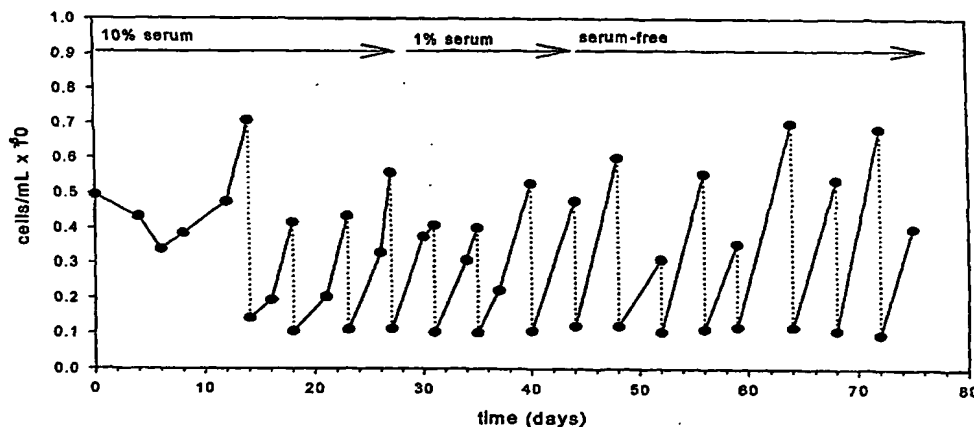
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[Continued on next page]

(54) Title: GLUTAMINE-AUXOTROPHIC HUMAN CELLS CAPABLE OF PRODUCING PROTEINS AND CAPABLE OF GROWING IN A GLUTAMINE-FREE MEDIUM



(57) Abstract: A glutamine-auxotrophic human cell transfected with an exogenous DNA sequence encoding a protein or an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a protein and an exogenous DNA sequence encoding a glutamine synthetase, wherein these exogenous DNA sequences are located on one or more than one DNA construct, said transfected cell capable of producing said protein and capable of growing in a glutamine-free medium.

**Declaration under Rule 4.17:**

- *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)*

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BEBBINGTON C R ET AL: "HIGH-LEVEL EXPRESSION OF A RECOMBINANT ANTIBODY FROM MYELOMA CELLS USING A GLUTAMINE SYNTHETASE GENE AS AN AMPLIFIABLE SELECTABLE MARKER" BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, vol. 10, no. 2, 1992, pages 169-175, XP000906901 ISSN: 0733-222X page 172, right-hand column, paragraphs 3,4; figures 3,5; table 2</p> <p style="text-align: center;">---</p> <p style="text-align: center;">-/--</p>	1-9

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZHOU WEICHANG ET AL: "Large scale production of recombinant mouse and rat growth hormone by fed-batch GS-NSO cell cultures." CYTOTECHNOLOGY, vol. 22, no. 1-3, 1996, pages 239-250, XP009012111 ISSN: 0920-9069 page 240, right-hand column, last paragraph -page 242, left-hand column, paragraph 2 ---	1-9
X	ZHOU W ET AL: "Fed-batch culture of recombinant NSO myeloma cells with high monoclonal antibody production" BIOTECHNOLOGY AND BIOENGINEERING, INTERSCIENCE PUBLISHERS, LONDON, GB, vol. 55, 1997, pages 783-792, XP002170348 ISSN: 0006-3592 page 784, right-hand column, paragraph 3 -page 785, left-hand column, paragraph 2 ---	1-9
X	WO 97 08307 A (KIM KEE WON ;KIM SUN YOUNG (KR); KIM TAE HAN (KR); LEE SUN YOUNG () 6 March 1997 (1997-03-06) page 21, line 2 -page 23, line 7; figure 8 ---	1-9
X	PU H ET AL: "RAPID ESTABLISHMENT OF HIGH-PRODUCING CELL LINES USING DICISTRONIC VECTORS WITH GLUTAMINE SYNTHETASE AS THE SELECTION MARKER" MOLECULAR BIOTECHNOLOGY, TOTOWA, NJ, US, vol. 10, 1998, pages 17-25, XP002923576 ISSN: 1073-6085 page 18, right-hand column, paragraph 2 ---	1,7-9
A	WO 99 05267 A (BRANDT MICHAEL ;FRANZE REINHARD (DE); PESSARA ULRICH (DE); BOEHRIN) 4 February 1999 (1999-02-04) examples 1,5-7; table 1 -----	1-9

INTERNATIONAL SEARCH REPORT

International patent family members

International Application No

PCT/EP 03/00454

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9708307	A	06-03-1997	AU 6756396 A EP 0851917 A1 JP 11502719 T WO 9708307 A1 KR 274225 B1	19-03-1997 08-07-1998 09-03-1999 06-03-1997 15-12-2000
WO 9905267	A	04-02-1999	AU 754619 B2 AU 8978698 A AU 737605 B2 AU 9067498 A BR 9811031 A BR 9811542 A CN 1265143 T CN 1265144 T WO 9905267 A1 WO 9905268 A1 EP 0986644 A1 EP 1000154 A1 JP 2001511342 T JP 2001511343 T TR 200000140 T2 TR 200000175 T2 US 2002164792 A1 US 6548296 B1 US 6395484 B1 US 6391633 B1 US 6555373 B1 US 2002110913 A1 ZA 9806515 A ZA 9806516 A	21-11-2002 16-02-1999 23-08-2001 16-02-1999 08-08-2000 22-08-2000 30-08-2000 30-08-2000 04-02-1999 04-02-1999 22-03-2000 17-05-2000 14-08-2001 14-08-2001 22-05-2000 22-01-2001 07-11-2002 15-04-2003 28-05-2002 21-05-2002 29-04-2003 15-08-2002 24-01-2000 24-01-2000